L89: Entry 11 of 12 File: USPT Jul 19, 1994

DOCUMENT-IDENTIFIER: US 5330754 A
TITLE: Membrane-associated immunogens of mycobacteria

APD:
19920629

BSPR:
Very little information about the mycobacterial genome is available. Initially, basic studies were conducted to estimate the genome size, G+C content and the degree of DNA homology between the various mycobacterial genomes (Grosskinsky, et al., Infect. Immun., 57, 5:1535-1541 (1989); Garcia, et al., J. Gen. Microbiol., 132:2265-2269 (1986); Imaeda, T., Int. J. Sys. Bacteriol., 35, 2:147-150 (1985); Clark-Curtiss, et al., J. Bacteriol., 161 3:1093-1102 (1985); Baess, I. et al., B., Acta. Path. Microbial. Scand., (1978) 86:309-312; Bradley, S. G., Am. Rev. Respir. Dis., 106:122-124 (1972)). Recently, recombinant DNA techniques have been used for the cloning and expression of mycobacterial geners. Genomic DNA fragments of M. tuberculosis, M. leprae and some other mycobacterial species were used for the construction of lambda gtll phage (Young, et al., Proc. Natl. Acad. Sci., U.S.A., 82:2583-2587 (1985); Young, et al., Nature (London), 316:450-452 (1985)) or other vector-based recombinant gene libraries. These libraries were screened with murine monoclonal antibodies (Engers, et al., Infect. Immun., 48:603-605 (1985); Engers, et al., Infect. Immun., 51:718-720 (1986)) as well as polyclonal antisera and some immunodominant antigens were identified. The principal antigen among these being five 12, 14, 19, 65 & 71 kDa of M. tuberculosis (Young et al., Proc. Natl. Acad. Sci., U.S.A., 82:2583-2587 (1985); Shinnick et al., Infect. Immun., 55(7):1718-1721 (1987); Husson and Young, Proc. Natl. Sc. Acad., 84:1679-1683 (1987); and five 12, 18, 23, 36 & 65 kDa antigens of M. leprae (Young, et al., Nature (London), 316:450-452 (1985)). A few homologues of some of these antigens were also identified in some other mycobacterial species (e.g., BCG) (Yamaguchi et al., FEB 06511, 240:115-117 (1988); Yamaguchi et al., Infect. Immun., 57:283-288 (1989); Matsuo, et al., J. Bacteriol., 170, 9:3847-3854 (1988); Radford, et al., Infect. Immun., 56, 4:921-925 (1988); Lu, et al., Infect. Immun., 55, 10:2378-2382 (1987); Minden, et al., Infect. Immun., 53, 3:560-564 (1986); Horboe, et al., Infect. Immun., 52, 1:293-302 (1986); Thole, et al., Infect. Immun., 50, 3:800-806 (1985)). These antigens, however, are either intracellular or secreted molecules.

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L20: Entry 33 of 45 File: USPT Apr 28, 1992

DOCUMENT-IDENTIFIER: US 5108745 A
TITLE: Tuberculosis and legionellosis vaccines and methods for their production

APD:
19880816

ABPL:
Vaccines and methods for their use in producing effective immune responses in mammalian hosts subsequently exposed to infection by intracellular pathogens including Legionella pneumophila wherein extracellular products of the pathogens are used as vaccines. After selecting a target intracellular pathogen, extracellular products of the selected pathogen which stimulate strong lymphocyte proliferative responses in immune hosts are then utilized as vaccines to immunize subsequent mammalian hosts to the target intracellular pathogen.

BSPR:
The present invention relates in a broad aspect to vaccines against intracellular parasites. More specifically, the present invention relates to vaccines and methods for their use in producing an effective immune response in mammalian host animals subsequently exposed to infection by intracellular parasites such as Leoionella pneumophila wherein the extracellular products of such pathogens are used as vaccines rather than utilizing the surface components of such sequestered bacterial pathogens.

BSPR:
Most bacteria are harmless. In fact, many are beneficial to man and to other mammalian animals. However, certain bacteria are able to grow and to spread in human and mammalian tissues. One class of such virulent organisms of particular interest is the intracellular pathogens. This category of virulent pathogens multiplies within the cells of the infected host organism rather than extracellularly.

BSPR:
The broad classification of intracellular pathogens includes organisms that are major causes of morbidity and fatality world-wide. For example, intracellular pathogens are responsible for the estimated 10,000,000 new cases of tuberculosis per year in the world (approximately 25,000 per year in the United States), the approximately 3,000,000 deaths per year from tuberculosis, and the estimated 12,000,000 cases of leprosy. They are also responsible for the estimated 10,000,000 cases of American trypanosomiasis (Chagas disease). Additionally, intracellular pathogens also cause other important diseases including cutaneous and visceral leishmaniasis, listeriosis, toxoplasmosis, histoplasmosis, trachoma, psittacosis, Q-fever, and Legionellosis including Legionnaires' disease. Few vaccines are available against such intracellular pathogen engendered diseases. The only widely used vaccine is BCG vaccine against tuberculosis. BCG vaccine is a live bacterial vaccine of questionable efficacy used primarily in Europe.

BSPR:
Briefly, the immunization method of the present invention utilizes extracellular products from such intracellular pathogens as immunizing agents rather than components of the pathogens themselves. Once immunized, these extracellular bacterial products are recognized by the host's immune system which can mount an

effective immune response to subsequent infection by such pathogens.

BSPR:
In accordance with the teachings of the present invention effective mammalian vaccines against specific intracellular pathogens are produced by first selecting a target intracellular pathogen, identifying one or more extracellular products of the pathogen which stimulate strong lymphocyte proliferative responses in mammalian hosts which are immune to the target pathogen, and then immunizing hosts with the extracellular product.

BSPR:
An exemplary embodiment of the present invention utilizes the major secretory protein (MSP) of L. pneumophila. MSP is the major protein released into culture supernates during growth of L. pneumophila and induces a strong cell-mediated immune response in immunized mammals. MSP is easily obtained following growth of Legionella pneumophila in a laboratory broth.

BSPR:
Following purification, the MSP is administered, preferably through injection, to mammalian host animals either alone or with an adjuvant such as Freund's adjuvant or incomplete Freund's adjuvant. For example, immunization with purified MSP in complete Freund's adjuvant by subcutaneous injection followed by a second injection in incomplete Freund's adjuvant approximately 3 weeks later may be utilized. However, it is contemplated as being within the scope of the present invention to utilize a single administration of extracellular product where such compounds will induce an effective immune response to a target pathogenwith a single dosage.

BSPR:
Because it is believed that MSP and other secretory products of intracellular pathogens are released extracellularly by the infected host cells, the present invention enables the immune system of a vaccinated host 30 to detect pathogens sequestered in the host's cells. In this manner, the vaccinated host's immune system is able to activate an effective immune response to kill or inhibit the multiplication of the pathogen inside it. Equally important, antibodies directed against the extracellular secretory products do not induce uptake of the intracellular pathogen and therefore do not facilitate infection. This is particularly important for immunocompromised patients and organ transplant patients.

DEPR:
For example, following this strategy, MSP, the major secretory protein of L. pneumophila, was identified as being a molecule to which immune guinea pigs developed a very strong cell-mediated immune response. A simple three-step procedure for purifying large quantities of MSP was developed. Lymphocytes from immune animals were found to proliferate strongly in response to minute concentrations of MSP. Similarly,, immune animals also developed a strong cutaneous delayed-type sensitivity to MSP. Guinea pigs subcutaneously immunized with MSP were studied for immune responses and it was determined that the MSP immunized guinea pigs developed strong specific cell-mediated immune responses (lymphocyte proliferation and cutaneous delayed-type sensitivity) to the MSP and, most importantly, developed effective immunity to lethal aerosol challenge with L. pneumophila.

DEPR:
In two independent experiments, guinea pigs were immunized subcutaneously with MSP 40 ug in Complete Freunds followed three weeks later by 40 ug in Incomplete Freunds) or sham-immunized (controls) subcutaneouslyu (Complete Freunds only). Splenic lymphocytes were obtained and incubated without antigen, or with the extracellular proteins of mutant L. pneumophila Philadelphia 1, L. pneumophila Togus 1, or L. pneumophilsChicago 2 at the concentrations indicated. Simulation indices were calculated.

DEPR:
1--immunized pigs exhibited a marked proliferative response to extracellular

proteins of L. pneumophila Serogroups 1, 2, and 6 in comparison to control guinea pigs.

DEPR:
It should be noted that, as shown in FIG. 2, MSP from L. pneumophila Philadelphia 1 (Serogroup 1) shares common antigens with MSP-like molecules of the same apparent molecular weight from L. pneumophilaTogus 1 (Serogroup 2), and L. pneumophila Chicago 2 (Serogroup 6), and a mutant derived from L. pneumophila Philadelphia 1. Total membranes of wild-type L. pneumophila Philadelphia 1 (Lane A), ammonium sulfate precipitated extracellular proteins of mutant L. pneumophila Philadelphia 1 (Lane B), L. pneumophila Togus 1 in Serogroup 2 (Lane C), L. pneumophila Chicago 2 in serogroup 6 (Lane D) and purified MSP from wild-type L. pneumophila Philadelphia 1 in Serogroup 1 (Lane E) were separated by SDS-PAGE and electrophoretically transferred onto nitrocellulose paper. The blots were then incubated with a 1:500 dilution of antiserum from a guinea pig immunized with purified MSP from wild-type L. pneumophila Philadelphia 1. The antigen-antibody complexes were revealed histochemically, using alkaline phosphatase --conjugated goat anti-guinea pig IgG.

DEPR:
Those skilled in the art will appreciate further benefits of the present invention. For example MSP and other secretory or extracellular products are single types of molecules rather than whole bacteria, therefore less toxicity is likely to result from the vaccines of the present invention in contrast to known vaccines against intracellular organisms. Additionally, such extracellular products are easily obtained and purified and may also be produced synthetically through recombinant DNA technology and other techniques for the production of protein molecules known to those skilled in the art.

DEPR:
Those skilled in the art will appreciate that the present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof. In that the foregoing description of the present invention discloses only preferred embodiments thereof, it is to be understood that other variations are contemplated as being within the scope of the present invention. Thus, by way of example, and not of limitation, the extracellular products of other intracellular parasites may be utilized to practice the present invention. Accordingly, the present invention is not limited to the particular embodiments which have been described in detail herein. Rather, reference should be made to the appended claims as indicating the scope and content of the present invention.

DETL:
TABLE G

Concentration (ug/ml) of Stimulation Index (S.I.)* Exper- Sero- Extracellular Immunized Control S.I. Immunized/ iment Strain group Proteins Guinea Pigs Guinea Pigs S.I. Control

A Phil 1 1 20 12.8 4.0 3.2 (Mutant) 2 19.2 6.2 3.1 Togus 1 2 20 19.3 7.0 2.8 2 15.8 6.8 2.3 Chicago 2 6 20 20.2 7.6 2.7 2 11.7 6.4 1.8 B Phil 1 1 20 19.0 5.1 3.7 (Mutant) 2 17.0 6.9 2.5 Togus 1 2 20 21.4 7.0 3.1 2 14.0 6.9 2.5 Chicago 2 6 20 12.8 8.2 1.6 2 14.3 6.1 2.3

*S.I. = Mean skin reactivity (mm) mutant immunized guinea pigs/mean skin reactivity (mm) of control guinea pigs.

CLPR:
2. The method of claim 1 wherein said extracellular product is Legionella pneumophila major secretory protein.

CLPR:
4. A vaccinating agent for use in promoting an effective immune response to Lecionella pneumophila in mammals, said vaccinating agent comprising Legionella pneumophila 1 major secretory protein.

CLPR:
5. The vaccinating agent of claim 4 wherein said major secretory protein is denatured.

CLPR:
6. The vaccinating agent of claim 4 wherein said major secretory protein is cleaved into smaller subunits.

CLPR:
7. The vaccinating agent of claim 4 wherein said major secretory protein is produced from Legionella pneumophila culture supernate.

CLPR:
8. The vaccinating agent of claim 4 wherein said major secretory protein is synthetically produced.

CLPR:
10. A method for immunizing a human host against subsequent exposure to Legionella pneumophila, said method comprising the step of immunizing said host with Legionella pneumophilamajor secretory protein.

CLPR:
11. The method of claim 10 wherein said major secretory protein is denatured.

CLPR:
12. The method of claim 10 wherein said major secretory protein is cleaved into smaller subunits.

CLPR:
13. A method for producing human vaccine against Mycobacterium tuberculosis, said method comprising the steps of:

CLPR:
14. Vaccine produced according to the method of claim 13 wherein said extracellular product is Mycobacterium tuberculosis major extracellular protein.

CLPR:
15. Vaccine produced according to the method of claim 13 wherein said intracellular pathogen is Mycobacterium tuberculosis.

CLPR:
16. A method for immunizing a human host against subsequent exposure to Mycobacterium tuberculosis, said method comprising the step of immunizing said host with Mycobacterium tuberculosis major extracellular protein.

CLPR:
17. A vaccinating agent for use in promoting an effective immune response to Mycobacterium tuberculosis in humans, said vaccinating agent comprising Mycobacterium tuberculosis extracellular protein.

CLPV:
identifying at least one extracellular product of Legionella pneumophila which stimulates strong cell mediated immune responses in at least one mammalian host infected with or immune to Legionella pneumophila; and

CLPV:
determining a human protective immunity inducing effective amount of said extracellular product.

CLPV:
identifying at least one extracellular product of Mycobacterium tuberculosis which stimulates strong cell mediated immune responses in at least one mammalian host infected with or immune to Mycobacterium tuberculosis; and

CLPV:
determining a human protective immunity inducing effective amount of said extracellular product.

ORPL:
"Purification and Characterization of an Extracellular Protease of Legionella pneumophila", Lawrence A. Dreyfus and Barbara H. Iglewski, Infection and Immunity, Mar. 1986, pp. 736-743.

ORPL:
"In Vitro Production of an Extracellular Protease by Legionella pneumophila", Michael R. Thompson, Richard D. Miller and Barbara H. Iglewski; Infection and Immunity, Oct. 1981, pp. 299-302.

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L94: Entry 10 of 16 File: USPT Apr 28, 1992

DOCUMENT-IDENTIFIER: US 5108745 A
TITLE: Tuberculosis and legionellosis vaccines and methods for their production

APD:
19880816

CLPR:
14. Vaccine produced according to the method of claim 13 wherein said extracellular product is Mycobacterium tuberculosis major extracellular protein.

CLPR:
16. A method for immunizing a human host against subsequent exposure to Mycobacterium tuberculosis, said method comprising the step of immunizing said host with Mycobacterium tuberculosis major extracellular protein.

CLPR:
17. A vaccinating agent for use in promoting an effective immune response to Mycobacterium tuberculosis in humans, said vaccinating agent comprising Mycobacterium tuberculosis extracellular protein.

CLPV:
identifying at least one extracellular product of Mycobacterium tuberculosis which stimulates strong cell mediated immune responses in at least one mammalian host infected with or immune to Mycobacterium tuberculosis; and

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L94: Entry 1 of 16 File: USPT Sep 19, 2000

DOCUMENT-IDENTIFIER: US 6120776 A
TITLE: Diagnostic skin test for tuberculosis

PRAD:
19930702

BSPR:
MPT64 is a protein which is secreted and released from metabolizing mycobacteria, in particular mycobacteria from the tuberculosis complex.

DEPR:
Because MPT64 and MPT59 are secreted proteins, it is relevant to compare reactivity in GP's sensitized with living and killed bacteria. Groups of GP's were infected with M. tuberculosis, BCG or immunized with killed M. tuberculosis in oil and skin tested 3 weeks later. The results show that similar tuberculin reactions were induced by the different sensitizations (FIG. 4). Positive skin reactions to MPT64 and MPT59 were, however, absent in GP's immunized with killed M. tuberculosis, and as seen previously in BCG-vaccinated GP's.

ORPL:
Wiker, H.G., et al., "A Family of Cross-Reacting Proteins Secreted by Mycobacterium tuberculosis", Scand. J. Immunol. 36:307-319 (1992).

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End of Result Set

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L89: Entry 12 of 12 File: USPT Oct 31, 1978

DOCUMENT-IDENTIFIER: US 4123427 A
TITLE: Method for the purification of mycobacterial protein antigens and resulting product

APD:
19760727

BSPR:
Antigen 6 is present in many mycobacteria. It has been shown to share an antigenic determinant when prepared from M. tuberculosis with a major antigen of M. szulgai, and also to contain a separate, nonshared antigenic determinant [Daniel, T. M. and DeMuth, R. W.: Immunological Chemical Analyses of a Major Antigen of Mycobacterium szulgai. Journal of Infectious Disease, Vol. 135, p. 778-786]. Subsequent unpublished observations by this inventor have demonstrated similar occurrences of shared and specific antigenic determinants on the antigen 6 molecule of several species of mycobacteria. As purified by Daniel and Ferguson [Daniel, T. M. and Ferguson, L. E.: Purification and characterization of two proteins from culture filtrates of Mycobacterium tuberculosis H.sub.37 Ra strain. Infect Immun 1: 164-168, 1970] and designated by them as protein a.sub.2 antigen 6 is a protein with a molecular weight of 45,000 to 48,000. The alpha antigen of Yoneda and Fukui [Yoneda, M.and Fukui, Y.: Isolation, purification, and characterization of extracellular antigens of Mycobacterium tuberculosis. Am Rev Respir Dis 92 (suppl): 9-18, 1965] is probably the same antigen. They have demonstrated antigenic specificity and shared determinants for their alpha antigen in a similar fashion [Yoneda, M., Fukui, Y., and Yamanouchi, T.: Biken J 8: 201-223, 1965].